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Confirmation of association between the e4 allele of apolipoprotein E and Alzheimer's disease

M Liddell, J Williams, A Bayer, F Kaiser, M Owen

Abstract

The Apo E genotype of 86 patients with Alzheimer's disease (AD) and 77 age matched controls was determined by digestion of Apo E PCR products with the restriction enzyme *CfoI*. The frequency of the e4 allele was significantly increased in the patient group (0.33) as compared with controls (0.12). This effect was seen in patients with a family history and in sporadic cases. The odds ratio in homozygotes for the e4 allele was 11.24 (95% confidence interval 2.45-51.50). There was no relationship between age of onset and Apo E genotype. There was no linkage disequilibrium between the apolipoprotein E locus and a *TaqI* polymorphism at the Apo CII locus, and no allelic association between Apo CII and AD.

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Alzheimer's disease is the commonest cause of presenile and senile dementia. Its incidence increases with advancing age, such that by the ninth decade as many as 36% of people may be affected. Although the characteristic neuropathological changes are fairly uniform from case to case, the condition is aetiologically heterogeneous.

Most recent molecular genetic studies have focused upon early onset "familial Alzheimer's disease" (FAD) because its pattern of inheritance suggests simple autosomal dominant transmission. In some families FAD is associated with mutations within exons 16 and 17 of the amyloid precursor protein (APP) gene on chromosome 21¹⁻⁴ and most of the remainder have been linked to a locus on the long arm of chromosome 14.⁵⁻⁸ The so-called "Volga-German" FAD kindreds are not linked to either APP or chromosome 14,⁹ thereby implicating the existence of at least a third locus. Thus, even apparently genetically simple FAD is genetically heterogeneous.

The genetics of common, late onset, and sporadic AD is more complicated, and this disorder is almost certainly both aetiologically and genetically heterogeneous. Complex segregation analysis suggests the possibility of one or two genes of major effect acting on a polygenic-multifactorial background.⁹ Putative susceptibility loci have been identified using non-parametric linkage methods on chromosomes 19 and 21.^{10,11}

Evidence for a susceptibility locus on chromosome 19 has also come from association studies. Schellenberg *et al*¹² reported an association between familial AD and the smaller

allele (A²) of a *TaqI* RFLP of the apolipoprotein CII gene on the long arm of chromosome 19. More recently, Roses' group have shown a strong association between the e4 allele of apolipoprotein E in both familial and sporadic late onset AD.^{13,14} We have attempted to replicate these two findings. Although we were unable to show an association between AD and the *TaqI* polymorphism at the Apo CII locus, we have been able to replicate the finding of a strong association between AD and the e4 allele of Apo E.

Methods

The patient group consisted of 95 subjects with AD (44 males and 51 females): 34 were obtained from the Cardiff Memory Clinic; 28 were the first sampled members of families with more than one case of late onset AD; and 33 were recruited from local psychogeriatric hospitals. The mean age of the patient group was 73.5 years (SD 10.1) with a mean age of onset of 67.5 years (SD 9.3). The majority of patients had an age of onset greater than 65 years (n = 56). Patients met the NINCDS criteria for probable AD.¹⁵ An age matched control group consisting of 86 subjects was obtained from the community with the help of a local general medical practice who identified elderly non-demented persons from their practice records. Potential controls were screened with the Mini-Mental State examination¹⁶ and included if they scored 28 or higher. The mean age of the control group was 73.2 years (SD 6.07). All patients and controls were white.

With the approval of the local ethics committee and the consent of the patient's nearest relative, a sample of venous blood, anticoagulated with EDTA, was taken and DNA extracted from the white cells by a standard procedure. Digestion of the DNA and Southern blotting were also carried out by standard procedures. The Apo CII cDNA probe used was a 440 bp cDNA insert,¹⁷ which detects allelic fragments of 3.8 kb and 3.5 kb, designated A1 and A2 respectively, after digestion with *TaqI*. PCR analysis of apolipoprotein E genotypes was carried out by the method of Wenham *et al*.¹⁸

Differences in allele and genotype frequencies were analysed by the method of Woolf.¹⁹ The observed and expected genotype frequencies were compared in order to ensure that the loci were in Hardy-Weinberg equilibrium (HWE) using a χ^2 goodness of fit test. For the Apo E polymorphism, alleles 2 and 3 were collapsed because of small cell sizes. Haplotype frequencies were estimated according to a

Department of Psychological Medicine, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN, UK
M Liddell
J Williams
F Kaiser
M Owen

Department of Medical Genetics, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN, UK
M Liddell
M Owen

Department of Geriatric Medicine, University of Wales College of Medicine, West Wing, Cardiff Royal Infirmary, Newport Road, Cardiff CF2 1SZ, UK
A Bayer

Correspondence to Dr Liddell.

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maximum likelihood procedure.²⁰ The significance of linkage disequilibrium (D) was tested by a likelihood ratio test.

Results

APO CII ASSOCIATION STUDY

Results were obtained for 78 controls (38 males) and 89 patients (43 males) and are shown in table 1. The frequency of the smaller 3.5 kb allele in the control group was 0.52 and in the patient group 0.53. Forty seven of the patient group were known to have affected first degree relatives and, considering this subgroup, the frequency of the A2 allele was 0.57. This difference was not statistically significant ($\chi^2 = 1.31$, $df = 1$, $p = 0.25$). The observed distributions of genotypes were close to the values expected on the basis of Hardy-Weinberg equilibrium in both the patients ($\chi^2 = 0.2$, $df = 1$, $p = 0.65$) and controls ($\chi^2 = 3.16$, $df = 1$, $p = 0.08$).

APO E ASSOCIATION STUDY

Apo E genotyping was successful in 77 controls (38 males) and 86 patients (43 males) and

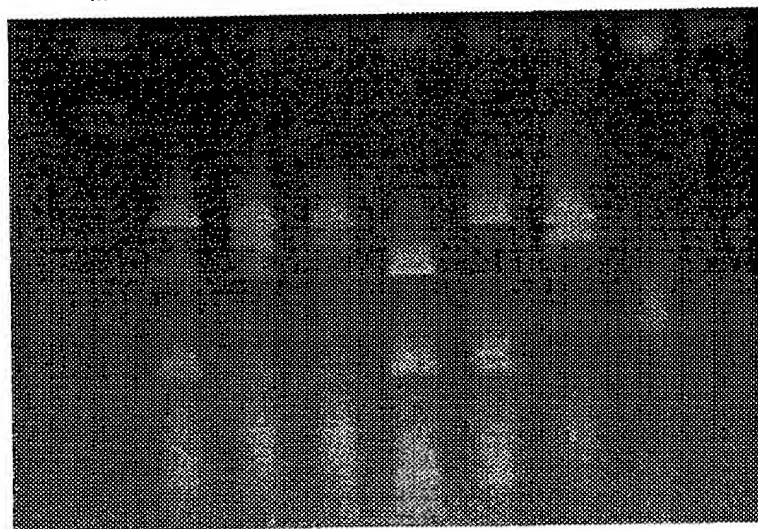
Table 1 Distribution of Apo CII TaqI RFLP genotypes in AD patients and age matched controls

	A ₁ A ₁	A ₁ A ₂	A ₂ A ₂
Patients (n = 89)	19	45	25
Controls (n = 78)	14	47	17

Table 2 Apolipoprotein E genotype distribution in AD patients and age matched controls

	Genotype					
	3/3	3/2	3/4	4/4	2/2	2/4
Patients (n = 86)	38	6	25	16	1	0
Controls (n = 77)	46	13	13	2	1	2

M 1 2 3 4 5 6 7 M



The appearance of the six possible Apo E genotypes after digestion of PCR amplified Apo E DNA with CfoI and electrophoresis through 10% polyacrylamide (after Wenham et al¹⁸). The tracks are identified as follows. Tracks M, DNA size markers; track 1, Apo E 3/4 genotype; track 2, Apo E 2/4 genotype; track 3, 2/3 genotype; track 4, Apo E 4/4 genotype; track 5, Apo E 3/3 genotype; track 6 Apo E 2/2 genotype; track 7, undigested PCR amplified DNA.

the results are shown in table 2. The figure illustrates the appearance of the six possible Apo E genotypes after digestion of Apo E PCR products with CfoI and electrophoresis through 10% polyacrylamide. In the control group the frequencies of e2 (0.11), e3 (0.77), and e4 (0.12) alleles did not differ markedly from those found previously in population surveys.²¹⁻²³ In contrast, the prevalence of the e4 allele was markedly increased in the patients (0.33) as compared to the control group (0.12) and this was highly significant ($\chi^2 = 18.4$, $df = 1$, $p = 0.00002$; OR = 3.5; 95% confidence interval 2-6.3. The risk of AD in e4 homozygotes (OR = 10.67, 95% confidence interval 2.3-48.8) was greater than in e4 heterozygotes (OR = 2.22, 95% confidence interval 1.1-4.7) when compared with those without the e4 allele. However, the difference between e4 homozygotes and heterozygotes did not achieve statistical significance ($\chi^2 = 3.7$, $df = 1$, $p = 0.055$; OR = 4.8; 95% confidence interval 1-23.9). Genotype frequencies in the control group were close to those expected on the basis of HWE ($\chi^2 = 0.51$, $df = 1$, $p = 0.5$). Significant departure from HWE was observed in the patients ($\chi^2 = 9.97$, $df = 1$, $p = 0.002$), reflecting the relative excess of e4 homozygotes in this group.

A one way analysis of variance comparing age of onset in patients with no, one, or two copies of the e4 allele found no significant effect (F ratio = 1.95, $df = 2$, $p = 0.15$). On the other hand, there appeared to be some effect of family history on the prevalence of the e4 allele, the frequency of the e4 allele being 0.4 in the presence and 0.26 in the absence of a family history of AD, although this trend was not statistically significant ($\chi^2 = 2.3$, $df = 1$, $p = 0.13$).

LINKAGE EQUILIBRIUM BETWEEN THE APO CII AND APO E LOCI

Using the data in table 3 it is possible to ascertain unambiguously 134 patient and 122 control Apo E-Apo CII haplotypes. In the patient group, there appears to be a slight excess of the e4 A2 haplotype, but the distribution skew was not significant at the 5% level ($\chi^2 = 4.55$, $df = 2$, $p = 0.1$). There was no suggestion of linkage disequilibrium occurring in the control group ($\chi^2 = 1.83$, $df = 2$, $p = 0.4$).

Discussion

We have replicated the original observation of Strittmatter et al¹³ of an association between

Table 3 Distribution of Apo CII (A) and Apo E (E) genotypes in AD patients and controls

Genotypes Apo E	Patients			Controls		
	Apo CII			Apo CII		
	A ₁ A ₁	A ₁ A ₂	A ₂ A ₂	A ₁ A ₁	A ₁ A ₂	A ₂ A ₂
E ₂ E ₂	—	1	—	—	1	—
E ₂ E ₃	1	1	4	2	6	4
E ₂ E ₄	—	—	—	1	—	1
E ₃ E ₃	12	16	7	10	29	7
E ₃ E ₄	3	11	7	1	8	3
E ₄ E ₄	1	9	6	—	1	1

the $\epsilon 4$ allele of apolipoprotein E and AD. Our data also suggest that the risk of AD may be greater in $\epsilon 4$ homozygotes than in heterozygotes. The frequency of the $\epsilon 4$ allele in our patients is not as strong as in the first study (0.33 v 0.50). A plausible explanation for this difference is that the cases studied by Strittmatter *et al.*¹³ were all from multiply affected families. In contrast, our patient group, although enriched for a positive family history, contained almost 50% of apparently sporadically occurring AD. In fact, Saunders *et al.*¹⁴ have found that the frequency of the $\epsilon 4$ allele in probable sporadic AD was 0.36, a figure which is closer to our own. Interestingly, these workers also found that the $\epsilon 4$ frequency in 176 cases of necropsy proven AD was, at 0.4, higher than the rate for clinically diagnosed probable AD. This raises the possibility that diagnostic inaccuracy may have reduced the $\epsilon 4$ frequency in our clinically diagnosed series. However, the trend in our data suggests that the $\epsilon 4$ allele frequency is higher in the family history positive cases and this would be in keeping with the presumptive increase in genetic loading expected in AD kindreds.

Our failure to show an association between AD and the small allele of the Apo CII *TaqI* RFLP is at odds with the work of Schellenberg *et al.*¹² It is possible that the increased familial loading in their sample may, in part, account for this difference. Another possibility is that there is an age effect on the allele frequency of this RFLP such that bearers of the small *TaqI* RFLP are more likely to reach advanced age. Thus, the fact that our control group was age matched, whereas that of Schellenberg *et al.* was not, may explain why the frequency of the smaller allele was larger in our controls (0.53) than in the earlier study (0.39). Inadequate digestion of the DNA by *TaqI* in our study would seem to be an unlikely explanation for the differences in Apo CII *TaqI* RFLP allele frequencies between the two studies, since we obtain an excess of the smaller, therefore digested, restriction fragment.

The fact that we were unable to show linkage disequilibrium between alleles of the Apo CII and Apo E genes is in keeping with the later work of Houlston *et al.*,²⁴ although in view of the data trend in the patient group, it may well be that a larger patient series would show linkage disequilibrium between the two loci in AD patients.

The contribution made by apolipoprotein E to the pathogenesis of AD is unclear. Of course it is logically possible that the Apo E gene merely acts as a neutral marker for some other gene with which it is in linkage disequilibrium and which predisposes to AD. However, a number of lines of evidence point to the direct involvement of apolipoprotein E in the pathogenesis of AD. Like APP, it is known to be a neuronal stress protein, because its synthesis is greatly increased following injury to the nervous system.²⁵⁻²⁸ Apolipoprotein E has also been shown to bind to amyloid deposits in AD and Creutzfeldt-Jakob disease, as well as to intracellular neurofibrillary tangles.²⁹ Recent work suggests that it may facilitate the deposi-

tion of A4 peptide as amyloid. Strittmatter *et al.*¹³ have convincingly shown that apolipoprotein E binds avidly to synthetic A4 peptide and have gone on to show that the apolipoprotein $\epsilon 4$ form, at physiological pH, binds more rapidly to β amyloid peptide than the $\epsilon 3$ form.^{13,30} In another study Schmechel *et al.*³¹ have shown that AD brains from patients with one or two $\epsilon 4$ alleles have increased amyloid deposition as compared to brains from those who do not possess this allele. These findings suggest that apolipoprotein $\epsilon 4$ might increase the rate at which amyloid deposits accumulate. If this is correct, then it is perhaps surprising that our data, and that of Saunders *et al.*,¹⁴ do not show a relationship between $\epsilon 4$ genotype and age of onset of AD. However, this may have occurred as a result of ascertainment biases. Indeed, a more recent study of 42 families with late onset AD³² found that mean age of onset decreased from 84 to 68 years with increasing number of $\epsilon 4$ alleles.

Whatever the role apolipoprotein E plays in the pathogenesis of AD, it seems that there is an increased prevalence of the $\epsilon 4$ allele in patients with AD. The prevalence may be increased further in family history positive cases. We estimate that considering the patient group as a whole, possession of the $\epsilon 4$ allele increases the risk of AD by some 3.5-fold, and the relative risk in $\epsilon 4$ homozygotes may be as high as 10.

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